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COLORIMETRIC DETERMINATION OF SULFUR DIOXIDE IN FROZEN SHRIMP

By

Masamichi NAKAMURA*, Naoko BABA*, Tadayoshi NAKAOKA*, Yutaka WADA*, and Toshiharu KAWABATA**

Synopsis

NAKAMURA, M., BABA, N., NAKAOKA, T., WADA, Y. (Kanagawa Pref. P.H. Lab., Asahi ku, Yokohama 241) and T. KAWABATA (National Institute of Health, Shinagawa ku, Tokyo 141) Colorimetric determination of sulfur dioxide in frozen shrimp. Bull. Kanagawa P.H. Lab. No. 5, 23-28 (1975)

Comparative studies have been undertaken to determine sulfur dioxide (SO_2) residues in frozen shrimp samples between the microdiffusion method described in the Standard Methods of Analysis for Hygienic Chemists (1974) issued by the Pharmaceutical Society of Japan and the Official Method for estimating SO_2 residues in frozen shrimps being settled under the Food Sanitation Low of Japan (1973). As to the extractant, it was found that the Na-tetrachloromercurate used in the Official Method was superior than the Na-bicarbonate employed in the Standard Method in Point of recovery.

We have developed a rapid and simple colorimetric determination of SO_2 without microdiffusion process in which the *P*-rosaniline reagent is being added directly to the Na-tetrachloromercurate extract of a shrimp sample.

When the Official Method was compared with the direct colorimetric method on the shrimp samples which have been added with various amounts of SO_2, it was found that the former method gave recoveries of fairly high consistency, while the latter showed higher recoveries with larger deviations. As far as the accuracy is concerned, it can be concluded that the Official Method was much more accurate than the direct colorimetric method. The latter, however, seemed to be of practical value for determining a large number of samples in the routine analysis.

Foreword

The volume of frozen shrimp imported by Japan from Southeast Asia and South America has increased sharply in recent years.

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However, as shrimp turns rapidly black after the catch because of the action of oxygen[1], sulfites are widely used in numerous countries such as the US, Canada and South America as they are reputed to prevent blackening[2] and by way of consequence to prevent drops in commercial value. The allowable volume of residual sulfurous acid in shrimp has so far been set by Japan at 30 ppm or less. However, by the notification No. 98 of April 28, 1973 from the Japanese Ministry of Health & Welfare the volume as residual SO_2 in shelled shrimp was set at 100 ppm or less.

There are several methods of performing the quantitative determination of sulfurous acid in foodstuffs, one of them being the AOAC method[3]. Four types of quantification methods (called hereafter "health test methods") are adopted as testing method for health protection purposes by the Pharmaceutical Society of Japan. All these methods are used for the quantitative determination of sulfurous acid in vegetable food products such as wine, dried gourd shavings, dried apricots. They cannot be used for animal foodstuffs such as shrimp as the addition-recovery rates are poor (when adding 50 ppm of SO_2, the rate is about 30% with the oxidation method, about 35% with the iodine method, about 52% with the colorimetric method and about 60% with the microdiffusion method). Tsukuda et al[5] proposed to use sodium tetra- p. 24
chloromercurate (abbreviated hereafter as TCM) to quantify deproteinized extracts by the microdiffusion - P-rosaniline colorimetric method of Ishisaka et al[6]. An improvement of this method was announced in the May 24, 1973 enforcement notification (Environment and Food No. 110) issued by the Environmental Health Bureau Chief, at the Ministry of Health and Welfare. In the present study, we compared the micro-diffusion method (called hereafter "microdiffusion-standard method")

presented in the health test method for frozen shrimp and the method based on the enforcement notification (called hereafter the micro-diffusion-official method), and we report on the results obtained in our examinations of a simpler direct colorimetric method.

Quantitative determination of residual sulfurous acid in frozen shrimp

[Reagents]

1 Absorption fluid[4]: (sodium tetrachloromercurate[7], Na_2HgCl_4 (0.1M) abbreviated hereafter as TCM absorption fluid): prepared according to the method registered in the Health Tests Law.

2 Extractant: prepared by diluting the above TCM absorption fluid 4 times with water.

3 P-rosaniline-formaldehyde solution: prepared according to the method registered in the Health Tests Law.

4 Sulfurous acid standard solution: prepared according to the method registered in the Health Tests Law. However, to prepare the standard solution for the direct colorimetric method, we diluted with water and not with extractant.

Sulfurous acid standard solution 1 mL = 1 μg SO_2

5 Mercuric chloride saturated solution: We put 7 g of mercuric chloride in 100 mL of water and stirred thoroughly.

[Preparation of the test solutions]

After thawing the frozen shrimps, we took the lowest possible number of individuals or 1 specimen, washed their surface gently with running water, removed the shell, chopped the meat and mixed thoroughly

to prepare samples. We measured carefully 10 g of sample, added 70 mL of extraction fluid and ground for 1-2 min with an homogenizer. Thereafter, we transferred the ground sample to a 100 mL volumetric flask, added saturated mercuric chloride solution and brought to 100 mL. After shaking vigorously, we filtered (Toyo filter paper No. 5C) and used the filtrate as test solution.

[Quantitative Determination]

1 Microdispersion - standard method: Performed according to the Health Tests method

2 Microdispersion - official method: The quantitative determination procedure under the conditions hereafter follows the Health Tests method

inner chamber fluid: 1 mL of TCM absorption fluid

outer chamber fluid: 1 mL of test fluid and 0.2 mL of 25% phosphoric acid

sealant: phosphoric acid (85%) or white vaseline

dispersion time: 90 min (20-40°C)

After the dispersion, inner chamber fluid is put with a Komagome pipette in a graduated stoppered test tube and the inner chamber is washed with TCM absorption fluid. The washing fluids are combined and the TCM absorption fluid is added so that the total volume is brought to 5 mL. We added 1 mL of P-rosaniline - formaldehyde solution, mixed by shaking vigorously and left standing for 35 min at 20-25°C. We measured the absorbance of the obtained colored fluid around the wavelength of 560 mμ, and called this value A. We took 5 mL of sulfurous acid standard solution, added 1 mL of P-rosaniline - formaldehyde solution,

obtained coloration by the same procedure as for the test solution. We called As the absorbance obtained here.

We used for the control solution the same procedure as for the absorption fluid. The sulfurous acid (SO_2) content in the samples is found through the following computation formula or through a previously established calibration curve

$$\text{Sulfurous acid } (SO_2 \text{ ppm}) = \frac{A}{As} \times 5 \times \frac{V}{W}$$

W : Volume of sample used (g)

V : dilution multiple

3 Direct colorimetric method

We put 1 mL of test fluid in a graduated stoppered test tube, added water so as to have a total volume of 5 mL. We added 1 mL of P-rosaniline - formaldehyde solution, shook vigorously, left standing for 35 min at 20-25°C and measured around the 550 mμ wavelength the absorbance of the obtained colored solution. We called this the value A_1. We proceeded separately in the same way with a sample prepared by adding 1 mL of sulfurous acid solution (5 μg/mL) to 1 mL of test solution and denoted the absorbance by A_2. For the control solution, we added 5 mL of water to 1 mL of sample solution, and performed the quantitative determination through the following computation formula or from a previously established calibration curve. Since the calibration curve is not a straight line going through the point of origin in the quantitative determination, the way of finding the quantity from the calibration curve is strictly accurate. However to simplify the quantitative determination, one uses a method based on the following one-point correction method.

$$\text{Sulfurous acid } (SO_2 \text{ ppm}) = \frac{A_1}{A_2 - A_1} \times 5 \times \frac{V}{W}$$

W : Volume of sample collected (g)

V : dilution multiple

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Test results and discussion

(1) Choice of extraction fluid and examination of the extractant volume

Although SO_2 is an unstable substance, stable substances are said to be generated when SO_2 forms complex ions with TCM[8]. These complex salts are said to be stable even after 24 h. This is why we examined whether a TCM solution was a suitable extractant.

Fig. 1 shows the relationship between the TCM concentrations and the extraction rates.

It was shown that the extraction rate of TCM fluid was more than 90% with 0.02M and about 100% with more than 0.025M. This is why we decided to use 0.025 M TCM solution as extractant.

Fig. 2 shows the results of our examinations regarding the extractant volumes.

We discovered that an extraction rate of about 100% is obtained when extracting 10 g of sample with 50 mL of TCM extractant. p. 26

(2) Examination of the volume of saturated mercuric chloride solution added.

We performed the following tests to examine the effect of the addition volumes of the saturated mercuric chloride solution used as deproteinization agent upon the recovery rate of SO_2.

That is, we crushed thoroughly in a mortar shrimp (shelled) not treated with sulfite and put 10 g of the sample in 5 separate homogenizer cups. We added 700 μg of SO_2 to every cup, mixed thoroughly with a glass pestle, added 50 mL of extractant and blended for 1-2 min with

an homogenizer. Thereafter, we added 40, 20, 10 and 0 mL of saturated mercuric chloride solution to the cups and while washing with water, put the solutions in volumetric flasks, bringing the volumes to 100 mL. We filtered after shaking vigorously. We took 1 mL of each filtrate and measured the SO_2 by the microdiffusion method. The results are shown in Fig. 3.

The above test results show a 100% yield rate obtained with 20 mL or more of saturated mercuric chloride solution. It is believed that in this case the mercuric chloride combines with the proteins or that the recovery rate increases because of the separation of the masked SO_2.

As for the amount of saturated mercuric chloride solution addition and the volume of extraction, we used 20 mL so as to keep the saturated mercuric chloride solution to the minimum necessary volume and used 70 mL of extractant.

(3) Examination of the calibration curve

We prepared the calibration curve of the diffusion method by adding various concentrations of SO_2 to the 0.1M-TCM solutions. With 1-12 μg, rectilinearity was shown. We prepared the calibration curve of the direct colorimetric method by adding various concentrations of SO_2 to 0.014M-TCM solutions. As a result rectilinearity was shown with 1-12 μg as in the case of the diffusion method. When adding various concentrations of SO_2, using distilled water instead of 0.014M-TCM solutions, the prepared calibration curve was practically the same as the calibration curve prepared with 0.014M-TCM solutions. So, to make things simpler, we used for the direct colorimetric method a calibration curve prepared with distilled water. Thereafter, we added various

concentrations of SO_2 to shrimp extractant with no SO_2 added so as to examine whether or not there was an effect of matters extracted from the shrimp upon the calibration curves. The calibration curves prepared with distilled water became parallel and we discovered that the matters extracted from the shrimp had no effect upon the SO_2 calibration curves.

(4) Relationship between the TCM solution concentration and the absorbance

When the same concentrations of SO_2 are added to the TCM solutions and to the mercuric chloride solutions, the absorbances were virtually the same. The absorbance tended to decline as the mercuric chloride concentration rose. We think therefore that the mercuric chloride concentration affects the absorbance. We showed this in Fig. 5.

(5) Addition-recovery tests with the various testing methods

We added various concentrations of sodium hydrogensulfite aqueous solution to ground shrimp, mixed thoroughly and left standing for 30 min. Thereafter we performed recovery tests according to the various measurement methods. The results are shown in Table 1.

With the microdiffusion method - the Standard Method, the recovery rate was 50-75%. With the microdiffusion method - the Official Method the recovery rate was about 90% or more, except with the addition of 300 mg. With the direct colorimetric method, the value shown was about 10% higher than with the latter. The reason is that the blank value was marked up at about 10%.

The standard directions of the microdiffusion method (Standard Method), of the microdiffusion method (Official Method) and of the

direct colorimetric method are respectively 6.0, 3.0, 6.0 with a 100 ppm addition of SO_2. Scattering is somewhat more pronounced with the direct colorimetric method.

(6) Examination of the interfering matters

If sulfur-containing compounds are copresent in the samples, they react against the P-rosaniline-formaldehyde solution and might interfere with the quantitative determination. We dissolved 8 types of sulfur-containing compounds in water viz. methylmercaptan, ethylmercaptan, methyl sulfide, ethyl sulfide, L-cysteine, L-cystine, methionine and taurine and examined the reactions against P-rosaniline-formaldehyde solutions. As shown in Table 2, methylmercaptan, ethylmercaptan, ethylsulfide and L-cysteine were positive. However, as these sulfur-containing compounds are treated with mercuric chloride at the time when the extractant is prepared, methylmercaptan, ethylmercaptan and L-cystein form insoluble mercaptides. Furthermore, the methyl sulfides can be eliminated as they form insoluble addition compounds. We thus discovered that these sulfur-containing compounds do not interfere with the present direct colorimetric method. However, it seems that the fact that the measurement values are somewhat higher than with the two other methods is due to interfering substances other than those presently examined as there is no microdiffusion operation which is a clean-up operation process.

(7) Quantitative determination of residual sulfurous acid in imported frozen shrimp

Tables 3 and 4 show the measurement results obtained by the microdiffusion method (Standard Method), the microdiffusion method (Official Method) and the present direct colorimetric method with regard

to unshelled and with regard to shelled shrimp from South America and from Southeast Asia. The lowest determination values were given by the microdiffusion method (Standard Method). The values were remarkably lower particularly as compared to the methods employed for unshelled shrimp.

It appears that frozen shrimp from South America is treated with sulfites, but shrimp from Southeast Asia does not seem to be treated with the above chemical.

Conclusion

When adding SO_2 to frozen shrimp, the recovery rate is conspicuously higher with the microdiffusion method (Official Method) and with the direct colorimetric method than with microdiffusion (Standard Method). When we measured thereafter by the direct colorimetric method, the values shown were somewhat higher than with the other methods in spite of some scattering. The operation is simple and one hour or less is enough to perform quantification. It seems therefore to be practical for screening tests when handling numerous specimens at the site.

In the present determination method, we used mercuric chloride as extractant and deproteinization agent. However the tests were conducted at a time when the waste water regulations were not very strict. There seems to be a need for research into deproteinization not carried out with mercuric chloride. The present direct colorimetric method was further tested by Iwaida et al[8] and found to be practical.

A summary of this article was presented at the 26th Meeting of the Japanese Food Health Science Society.

(received June 30, 1975)

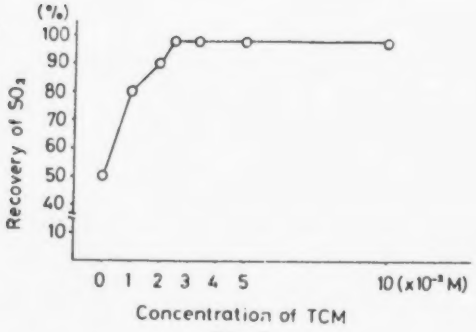

Fig. 1

Extraction rates of SO_2 from shrimp with TCM solution (500μg of SO_2 was added to 10 g of shrimp)

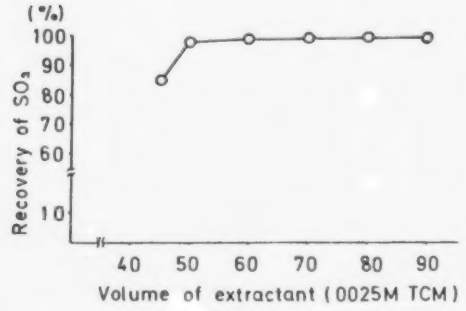

Fig. 2

Effect of volume of the extractant on the recovery of SO_2 added to shrimp
(700μg of SO_2 was added to 10 g of shrimp)

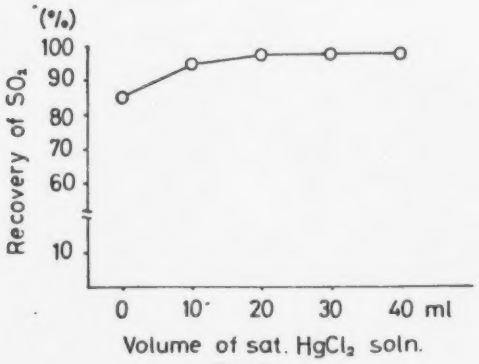

Fig. 3

Effect of volume of saturated $HgCl_2$ solution on the recovery of SO_2 added to shrimp
(700μg of SO_2 was added to 10 g of shrimp)

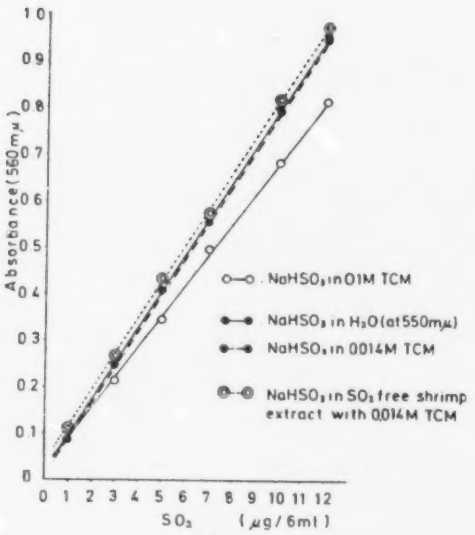

Fig. 4

Calibration curves for SO_2

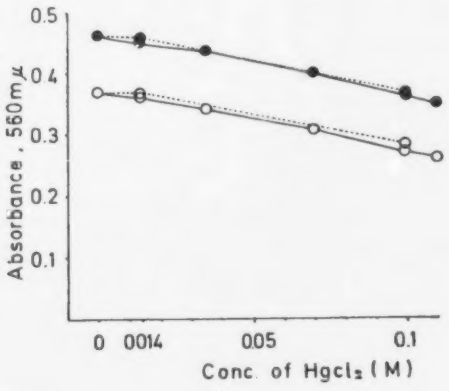

Fig. 5

Effect of $HgCl_2$ concentration on the absorbances of SO_2

- ●····● 7μg SO_2/6 ml in TCM (1$HgCl_2$: 2NaCl) soln.
- ●——● 7μg SO_2/6 ml in $HgCl_2$ soln.
- ○····○ 5μg SO_2/6 ml in TCM soln.
- ○——○ 5μg SO_2/6 ml in $HgCl_2$ soln.

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Table 1 Recovery tests of SO_2 added to the shrimp

Amount of SO_2 added to shrimp meat (mg/10g	Standard method*	Official method**	Direct method
300	50 ± 7.0	73 ± 4.7	85 ± 7.8
500	59 ± 6.4	89 ± 3.6	97 ± 7.8
700	68 ± 6.0	92 ± 3.6	103 ± 7.0
1000	75 ± 6.0	98 ± 3.0	105 ± 6.0

(Figures were obtained by 6 repeated experiments)

* Standard method settled by the Phamaceutical Society of Japan (Microdiffusion method)

** Official method settled by the Japanese Ministry of Health and Welfare (Microdiffusion method)

Table 2 Reaction between *P*-rosaniline formaldehyde solution and various S compounds

Name of S Compounds	Concentration	Absorbance (OD max without $HgCl_2$	with $HgCl_2$
CH_3SHNa	10^{-2} M	1.07 (560 mμ)	0.01
CH_3CH_2SH	10^{-2} M	0.60 (565 mμ)	0.01
$(CH_3)_2S$	saturated in H_2O	0.01	0.01
$(C_2H_5)_2S$	saturated in H_2O	0.49 (565 mμ)	0.01
L-Cysteine · HCl	10^{-2} M	0.72 (558 mμ)	0.01
L-Cystine	saturated in H_2O	0.01	0.01
Methionine	saturated in H_2O	0.01	0.01
Taurine	saturated in H_2O	0.01	0.01

Each sample (5 ml) was added with 1 ml of *P*-rosaniline-formaldehyde

Table 3 Concentration of SO_2 in the frozen shrimp samples imported from South America determined by 3 methods

Sample	Standard method	Official method	Direct method
1 L a	12.0ppm	44.0ppm	48.9ppm
b	9.0	21.0	23.0
2 L a	116.0	234.8	264.6
b	71.0	114.5	126.0
3 M a	4.2	20.5	26.0
b	3.8	10.5	15.8
4 M a	32.8	70.0	83.2
b	23.3	32.8	44.0
5 S a	70.0	142.0	158.1
b	53.7	72.4	77.2
6 S a	4.2	20.5	26.0
b	3.8	10.5	15.8

Note : L = Large size Av. wt 25-35g
M = Medium size : 10g
S = Small size : 7.5g
a = with shell
b = without shell

Table 4 Concentration of SO_2 in the frozen shrimp samples imported from Southeast Asian countries determed by 3 methods

Sample	Standard method	Official method	Direct method
1 L a	N. D.	N. D.	N. D.
b	N. D.	N. D.	N. D.
2 L a	N. D.	N. D.	N. D.
b	N. D.	N. D.	N. D.
3 M a	N. D.	N. D.	N. D.
b	N. D.	N. D.	N. D.
4 M a	N. D.	N. D.	N. D.
b	N. D.	N. D.	N. D.
5 S a	N. D.	N. D.	N. D.
b	N. D.	N. D.	N. D.
6 S a	N. D.	N. D.	N. D.
b	N. D.	N. D.	N. D.

N. D. = less than 0.1 ppm

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* Translator's note : probable reading only; not certain